

**U.S.S.N. 08/700,565  
GRUENBERG  
AMENDMENT**

B2  
At page 36, line 7, delete "copending, allowed, U.S. application Serial No. 08/506,173" and insert therefor —U.S. application Serial No. 08/506,173, now U.S. Patent No. 5,627,070—.

At page 36, line 14, delete "copending, allowed,".

At page 36, line 15, delete "08/506,173" and insert therefor —08/506,173, now U.S. Patent No. 5,627,070—.

B3  
At page 37, line 20, delete "allowed copending U.S. application Serial No. 08/506,173" and insert therefor —U.S. application Serial No. 08/506,173, now U.S. Patent No. 5,627,070—.

B4  
At page 54, line 28, delete "copending allowed U.S. application Serial No. 08/506,173" and insert therefor —U.S. application Serial No. 08/506,173, now U.S. Patent No. 5,627,070—.

B5  
At page 55, line 5, delete "copending allowed U.S. application Serial No. 08/506,173" and insert therefor —U.S. application Serial No. 08/506,173, now U.S. Patent No. 5,627,070—.

**IN THE CLAIMS:**

~~Please cancel claims 18-21 and 36-153 without prejudice or disclaimer.~~

Please add claims 154 to 196 as follows:

—154. ~~The method~~ of claim 2, wherein the expanded cells are predominantly Th1, Th2 or Th3 cells.—

B6  
—155. A method for generating clinically relevant numbers of regulatory T lymphoid cells for autologous cell therapy, comprising:

- Sub F5
- (a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;
  - (b) treating the cells to induce differentiation of mononuclear cells into regulatory T cells, wherein regulatory T cells are mononuclear cell that have the ability to control or direct an immune response, but do not act directly as effector cells in the response; and

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- Sub FS  
cont
- (c) contacting the resulting differentiated cells with one or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of regulatory cells for autologous cell therapy are generated.—

156. The method of claim 155, wherein cells are purified from the material.—

157. The method of claim 155, wherein the treating or contacting step occurs in the absence of exogenous cytokines.—

158. The method of claim 155, wherein the regulatory cells express a selected antigen.—

159. The method of claim 155, wherein the regulatory cells are CD4 + T-cells.—

160. The method of claim 155, wherein the regulatory cells are Th1, Th2 or Th3 cells.—

161. The method of claim 155, wherein the regulatory cells are CD8 + T-cells.—

162. The method of claim 155, wherein the cells are treated with either or both interferon- $\gamma$  and IL-2 to induce differentiation of Th1 cells.—

163. The method of claim 155, wherein the cells are treated with IL-4 with or without anti-gamma interferon antibodies and/or anti-IL-12 antibodies to cause differentiation into Th2 cells.—

164. The method of claim 155, wherein the proteins specific for cell surface proteins are one or more monoclonal antibodies specific for immune cell surface proteins.—

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—<sup>1165</sup>~~166~~. The method of claim ~~165~~, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more antigens selected from the group consisting of CD4, CD8, CD11a, CD27, CD28, CD44 and CD45RO.—

—<sup>1166</sup>~~167~~. The method of claim 155, wherein cell expansion is effected in a hollow fiber bioreactor.—

—<sup>1167</sup>~~168~~. The method of claim 155, wherein the cells are expanded to about  $10^9$  cells or greater.—

—<sup>1168</sup>~~169~~. The method of claim 155, wherein the cells are expanded to about  $10^{10}$  cells or greater.—

 —<sup>1169</sup>~~170~~. The method of claim 155, wherein the expanded cells are predominantly Th1, Th2, Th3 cells.—

—<sup>1170</sup>~~171~~. The method of claims 155, wherein the expanded cells are contained in a volume of one liter or less.—

—<sup>1171</sup>~~172~~. The method of claim 155, wherein the expanded cells are contained in a volume of about 500 mls or less.—

—<sup>1172</sup>~~173~~. The method of claim 155, wherein the expanded cells are contained in a volume of about 250 mls or less.—

—<sup>1173</sup>~~174~~. The method of claim 155, wherein the expanded cells are predominantly Th1-like, Th2-like, Th3-like, wherein:

Th1-like cells are cells that produce a majority of Th1 cytokines;

Th2-like cells are cells that produce a majority of Th2 cytokines; and

Th3-like cells are cells that produce a majority of Th3 cytokines.—

<sup>1174</sup>~~175~~. A method for generating clinically relevant numbers of regulatory Th1, Th2, Th3, Th1-like, Th2-like or Th3-like lymphoid cells for autologous cell therapy, comprising:

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- (a) collecting material comprising body fluid or tissue containing T lymphoid cells from a mammal;
- (b) treating the cells to induce differentiation of some of the mononuclear cells into Th1, Th2, Th3, Th1-like, Th2-like or Th3-like, wherein:

Th1-like cells are cells that produce a majority of Th1 cytokines;

Th2-like cells are cells that produce a majority of Th2 cytokines; and

Th3-like cells are cells that produce a majority of Th3 cytokines; and

- (c) contacting the cells with two or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of regulatory Th1, Th2, Th3, Th1-like, Th2-like or Th3-like

Th1, Th2, or Th3 lymphoid cells are generated.—

—176. The method of claim 175, wherein cells are either purified or purged from the material.—

—177. The method of claim 176, wherein the treating or contacting steps occur in the absence of exogenous cytokines.—

—178. The method of claim 176, wherein the regulatory cells are specific for a defined antigen.—

—179. The method of claim 175, wherein the regulatory cells are CD4 + T-cells.—

—180. The method of claim 175, wherein the regulatory cells are CD8 + T-cells.—

—181. The method of claim 175, wherein the cells are treated with either or both interferon- $\gamma$  and IL-2 to induce differentiation of Th1 cells.—

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<sup>181</sup>  
~~182.~~ The method of claim ~~175~~<sup>174</sup>, wherein the cells are treated with IL-4 with or without the presence of anti-gamma interferon monoclonal antibodies and/or anti-IL-12 monoclonal antibodies to cause the differentiation into Th2 cells.—

<sup>182</sup>  
~~183.~~ The method of claim ~~175~~<sup>174</sup>, wherein the proteins specific for cell surface proteins are one or more monoclonal antibodies specific for immune cell surface proteins.—

<sup>183</sup>  
~~184.~~ The method of claim ~~183~~<sup>182</sup>, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more of the following: CD4, CD8, CD11a, CD27, CD28, CD44, and CD45RO.—

<sup>184</sup>  
~~185.~~ The method of claim ~~175~~<sup>174</sup>, wherein cell expansion is effected in a hollow fiber bioreactor.—

<sup>185</sup>  
~~186.~~ The method of claim ~~175~~<sup>174</sup>, wherein the cells are expanded to an excess of  $10^9$  cells.—

<sup>186</sup>  
~~187.~~ The method of claim ~~175~~<sup>174</sup>, wherein the cells are expanded to an excess of  $10^{10}$  cells.—

<sup>187</sup>  
~~188.~~ The method of claim ~~175~~<sup>174</sup>, wherein the expanded cells are administered to a patient.—

<sup>188</sup>  
~~189.~~ The method of claims ~~175~~<sup>174</sup>, wherein the expanded cells are contained in a volume of about one liter or less.—

<sup>189</sup>  
~~190.~~ The method of claim ~~175~~<sup>174</sup>, wherein the expanded cells are contained in a volume of about 500 mls or less.—

<sup>190</sup>  
~~191.~~ The method of claim ~~175~~<sup>174</sup>, wherein the expanded cells are contained in a volume about 250 mls or less.—

<sup>191</sup>  
~~192.~~ The method of claim ~~175~~<sup>174</sup>, wherein the expanded cells are predominantly Th1, Th2 or Th3 cells.—

<sup>192</sup>  
~~193.~~ The method of claim ~~175~~<sup>174</sup>, wherein the expanded cells are predominantly Th1-like, Th2-like, Th3-like cells.—

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<sup>193</sup>  
~~194.~~ The method of claim <sup>174</sup>~~175~~, wherein the expanded cells are predominantly Th1 cells. —

<sup>194</sup>  
~~195.~~ The method of claim <sup>174</sup>~~175~~, wherein the expanded cells are predominantly Th2 cells. —

<sup>195</sup>  
~~196.~~ The method of claim <sup>174</sup>~~175~~, wherein the expanded cells are predominantly Th3 cells. —

<sup>196</sup>  
~~197.~~ The method of claim 16, wherein the expanded cells comprise tumor infiltrating lymphocytes (TIL) lymphokine activated killer (LAK) cells or cytotoxic T lymphocytes (CTLs). —

Please amend claims 1-7, 9-15 and 22-32 as follows:

<sup>197</sup>  
1. (Amended) A method for generating a high density of clinically relevant numbers of T lymphoid [immune] cells, comprising:

collecting material comprising body fluid or tissue containing mononuclear cells from a mammal; and

contacting, in the absence of exogenous interleukin-2, the material with [one] two or more activating proteins specific for cell surface proteins present on cells in the material and in an amount sufficient to induce ex vivo cell expansion, whereby the cells expand to clinically relevant numbers at a density of at least about 10<sup>9</sup> cells in a volume of about a liter [to clinically relevant numbers].

2. (Amended) The method of claim 1, wherein prior to the contacting step, the cells [in the material] are treated under conditions whereby *ex vivo* differentiation of some or all of the cells into selected regulatory immune cells is induced.

3. (Amended) The method of claim 1, wherein during the contacting step, the cells [in the material] are treated under conditions, other than addition of exogenous IL-2, whereby *ex vivo* differentiation of some or all of the cells into desired effector immune cells is induced.

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~~4. (Amended) The method of claim 1, further comprising purification of [wherein] the expanded cells [are purified].~~

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~~5. (Amended) The method of claim 2, further comprising purification of [wherein] the expanded cells [are purified].~~

~~6. (Amended) The method of claim 1, wherein the [immune] expanded cells are specific for a defined antigen.~~

~~7. (Amended) The method of claim 2, wherein the [immune] expanded cells are specific for a defined antigen.~~

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~~8. The method of claim 1, wherein the expanded cells are predominantly Th1, Th2 or Th3 cells.~~

~~9. (Amended) The method of claim 1, wherein the [immune] cells are activated *ex vivo* prior to the contacting step in the presence of either or both interferon- $\gamma$  and IL-2, whereby differentiation of Th1 cells [are] is effected.~~

~~10. (Amended) The method of claim 1, wherein the cells are activated *ex vivo* in the presence of [one or more of an agent selected from] IL-4 with or without the presence of [.] anti-gamma interferon and anti-IL-12 monoclonal antibodies to cause[, whereby] differentiation [of] into Th2 cells [is effected].~~

~~11. (Amended) The method of claim 1, wherein the proteins specific for cell surface [molecules] proteins are one or more monoclonal antibodies specific for immune cell surface proteins.~~

~~12. (Amended) The method of claim 11, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more of the following: CD4, CD8, CD11a, CD27, CD28, CD44 and CD45RO.~~

~~13. (Amended) The method of claim 1, wherein cell expansion is effected in a hollow fiber bioreactor.~~

~~14. (Amended) The method of claim 1, wherein the [immune] cells are expanded to an excess of  $10^6$  cells.~~

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15. (Amended) The method of claim 1, wherein the [immune] cells are expanded to an excess of  $10^{10}$  cells.

16. The method of claim 1, wherein the cells are effector [immune] cells.

17. The method of claim 1, wherein the cells are regulatory [immune] cells.

22. (Amended) A method for generating clinically relevant cell numbers of regulatory [immune] T lymphoid cells, comprising:

(a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) [treating] activating the cells to alter their cytokine production profile; and

(c) inducing cell proliferation and expanding the cells under conditions that produce high cell density of at least about  $10^9$  cells/liter [to a] and produce clinically relevant number of regulatory T lymphoid cells.

23. (Amended) The method of claim 22, wherein the [immune] T lymphoid cells with altered cytokine profile are purified [prior to infusion].

24. (Amended) The method of claim 22, wherein the [immune] T lymphoid cells with altered cytokine profile are specific for a defined antigen.

25. (Amended) The method of claim 23, wherein the [immune] T lymphoid cells with altered cytokine profile are specific for a defined antigen.

26. (Amended) The method of claim 22, wherein the [mononuclear] T lymphoid cells are activated [treated] to differentiate into Th1 or Th2 cells.

27. (Amended) The method of claim 22, wherein the resulting population of expanded cells [are] includes Th1-like or Th2-like cells.



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28. (Amended) The method of claim 22, wherein the [immune] T lymphoid cells are activated *ex vivo* in the presence of either or both interferon- $\gamma$  and IL-2, whereby cells differentiate [differentiation of] into Th1 cells [is effected].

29. (Amended) The method of claim 28, wherein anti-IL-4 [mAb is] monoclonal antibodies are also present during activation.

30. (Amended) The method of claim 29, wherein the [effector] cells are activated in the presence of IL-4 [or IL-4 and either or both] anti-gamma interferon antibodies and/or anti-IL-12 antibodies, whereby cells differentiate [differentiation of] into Th2 cells [is effected].

31. (Amended) The method of claim 22, wherein the cells are expanded in the presence of [one] two or more monoclonal antibodies [are included in the medium in which the mononuclear cells are expanded].

32. (Amended) The method of claim 31, wherein the monoclonal antibodies are specific for CD3 or CD2, combined with any combination of monoclonal antibodies specific for one or more of the following: CD4, CD8, CD11a, CD27, CD28, CD44 and CD45RO.

33. The method of claim 22, wherein the cells are expanded in a hollow fiber bioreactor.

34. The method of claim 22, wherein the cells are expanded to an excess of  $10^9$  cells.

35. The method of claim 22, wherein the cells are expanded to an excess of  $10^{10}$  cells.

REMARKS

A Petition for Extension of time and a check for the requisite fees for the Petition and for excess claims accompany this response. Any fees, including fees for additional claims, that may be due with this paper or with this application during its entire pendency may be charged to Deposit Account No.